Nature and Origin of Polymorphism in Feline MHC Class II *DRA* and *DRB* Genes[1]

Naoya Yuhki[2] and Stephen J. O'Brien

Transcripts of the MHC class II *DRA* and *DRB* gene homologues of the domestic cat (*Felis catus*) were cloned and sequenced to compare the pattern and process of *DR* gene divergence. Homologous *DRB* exon 2 sequences from 36 feral domestic cats throughout the world plus from three species of Felidae (tiger cat, Iriomote cat, and Geoffroy's cat) were also determined. Limited variation in the domestic cat *Feca-DRA* gene was observed, but abundant variation in the *Feca-DRB* gene was seen comprising 61 distinct *DRB* alleles. Phylogenetic analyses resolved at least five monophyletic feline *DRB* allelic lineages (*DRB1 to *5), which are clearly distinct from those of human (*HLA-DRB1* to 9 lineages), mouse (*H-2Eβ b, u, f*), and dog *DRB* alleles. Approximately 80% of individual cats contained three to six distinct *DRB* sequences, indicating that feline MHC maintains two to three *DRB* loci. Five cats had three *DRB* sequences in a single allelic lineage, indicating the occurrence of recent gene duplication of feline *DRB* genes. *DRB* sequences isolated from three exotic cats demonstrated close association with a particular domestic cat *DRB* lineage, suggesting that these allelic lineages are derived from common ancestral alleles that existed prior to the divergence of these feline species about 10 to 15 million years ago. Patterns of synonymous and nonsynonymous nucleotide substitution rates that occurred in Ag recognition sites (ARS) and nonrecognition (NAR) sites demonstrated a strong role of natural selection—positive selection for Ag recognition sites and negative selection for nonrecognition sites of feline *DRB* sequences—in the process of evolution of *DR* molecules.** *The Journal of Immunology,* **1997, 158: 2822–2833.**

The MHC encodes two categories of cell surface glycoproteins, which present short peptides to TCR (class I and class II molecules). Class I molecules, noncovalently associated with a β_2-microglobulin, are expressed on the surface of all somatic cells. Class I molecules mediate the destruction of virus-infected cells and tumor cells by presentation of foreign-processed peptides to TCRs of CD8-positive CD4-negative cytotoxic T cells. Class II α- and β-chains are expressed on APC, such as B cells, dendritic cells, macrophages, and epithelial cells. These cell populations present peptides to TCRs of CD4-positive CD8-negative Th cells and activate an immune system through the secretions of specific cytokines (1).

The domestic cat is an important study model for neurologic disorders and for inherited diseases in humans (2). Domestic cats are also endemic with three pathogenic RNA virus infections—feline leukemia virus (3), feline immunodeficiency virus (4), feline infectious peritonitis virus (5). In addition, the cat family Felidae comprises 38 species and provides an important model for the pattern and processes of adaptive evolution (6, 7).

The cat MHC is located on the centromeric region of feline chromosome B2 (8, 9) and showed a moderate to high polymorphism in its class I and class II molecules by several parameters such as graft experiments (10), serologic analysis of cat pedigrees (10), RFLPs of feline class I genes (11), and sequence analysis of cDNA clones (12–14). A close examination of sequences of feline class I cDNA clones from three feline species (domestic cat, cheetah, and ocelot) representing the major taxonomic lineages of Felidae revealed two primary factors for generation and maintenance of polymorphic class I sequences, which include 1) positive selection pressure in favor of polymorphism or high heterozygosity in the Ag recognition site, and 2) periodic intragenic (interallelic) and intergenic (interlocus) segmental exchanges among class I genes (13, 14). The short polymorphic sequence motifs (20 to 23 bp) found in Ag-binding sites of class I molecules and other sequence motifs throughout class I sequences in the domestic cat were also found in the sequences of exotic cat species, suggesting a generation of these motifs in a common ancestor of these modern feline species and a persistence of the motifs for a long period of time (10 to 15 million years)—a transpecies mode of evolution (14).

We present here an analysis of class II *DRA* and *DRB* gene homologue variations in domestic cats, including 61 distinct *DRB* exon 2 sequences sampled from eight countries and four continents. The results reveal genetic monomorphism of *DRA* and extensive polymorphism and phylogenetic lineages of *DRB* allelic sequences reflecting ancient origin and sequence divergence of these molecules during the radiation of Felidae.

Section of Genetics, Laboratory of Genomic Diversity, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702

Received for publication June 3, 1996. Accepted for publication December 13, 1996.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Tissue samples of endangered species were collected in full compliance with specific federal permits (CITES; Endangered and Threatened Species), issued by the U.S. Fish and Wildlife Service of the Department of Interior to the National Institutes of Health principal officer S. J. O'Brien.

[2] Address correspondence and reprint requests to Dr. Naoya Yuhki, Section of Genetics, Laboratory of Genomic Diversity, Bldg. 560 Rm. 2153, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick MD 21702-1201.

Materials and Methods

Animals

Samples from 37 feral domestic cat (*Felis catus*) were obtained from 11 locations in 8 countries, including Australia (AUS); Thailand (THAI); St. Petersburg, Russia (RUS STP); India (IND); Nicaragua (NCG); Central America (CAM); Buenos Aires, Argentina (ARG BA); Mendoza, Argentina (ARG MZ); Cordoba, Argentina (ARG CO); United Kingdom (UK); Maryland (USA MD); and Pennsylvania (USA PA) (see Table III). Genomic DNAs isolated from the tiger cat (*Leopardus tigrina*), Lti-6, and Geoffroy's cat (*Oncifelis geoffroyi*), Oge-1, and maintained in this laboratory were used for PCR analysis. PCR products of *DRB* exon 2-coding

Copyright © 1997 by The American Association of Immunologists

0022-1767/97/$02.00

region from the Iriomote cat (*Mayailurus iriomotensis*) were a gift from F. Shinyashiki, (Okinawa International University, Ginowan, Okinawa).

Construction of cDNA library and isolation of feline DRA and DRB cDNA clones

mRNAs were extracted and purified from spleen cells of an adult domestic cat (FCA 021). cDNA was synthesized by a modification of the method by Gubler and Hoffman (15), using a cDNA synthesis kit (Pharmacia Biotech, Piscataway, NJ). *Eco*RI adaptor DNA (Stratagene, La Jolla, CA) was ligated with cDNA, and after phosphorylation of the 5' end by T4 DNA kinase, these cDNA were ligated with *Eco*RI sites of λ zap II vector (Stratagene) and in vitro packaged using GIGAPACK GOLD II extract (Stratagene). A total of 0.8 million original plaques were screened by ^{32}P-labeled human *HLA-DRA* (16) and *DRB1* (17) cDNA as probes.

PCR, cloning, and sequencing PCR-amplified DNA segments

PCR was performed to amplify exon 2 region (238 bp excluding primer sequences) from 100 ng genomic DNAs using oligonucleotides for human *HLA-DRB* genes: *DRB*219, 5'-CCACACAGCACGTTTCT/CTG-3'; and *DRB*61A, 5'-CCGCTGCACTGTGAAGCT-3' (18). PCR reactions were prepared in 50-μl volumes including 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 10 pmol of each primer, and 2.5 U of Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN). Thermal cycles were performed in a MJ Research PTC-100 thermocycler under the following conditions: 94°C for 3 min followed by 30 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 1 min, and 72°C for 10 min after the last cycle. Ten microliters of the PCR products were electrophoresed onto 3% agarose gel (2% NuSieve and 1% SeaKem GTG agarose, FMC Bioproducts, Rockland, ME) with a 123-bp DNA ladder marker (Life Technologies, Gaithersburg, MD) and visualized by staining with ethidium bromide. Samples containing DNA segments with the predicted size were treated with 1 U of Klenow fragment of DNA polymerase I at room temperature for 20 min to polish the ends. These samples were transferred into centricon 100 (Amicon Danvers, MA), and 1.5 ml of TE (10 mM Tris-HCl (pH 8.0) 1 mM EDTA) buffer were added and centrifuged at 6500 rpm for 10 min. Seven microliters of the sample were used to ligate with 100 ng of *Eco*RV-digested pBS SK$^+$ vector (Stratagene) using 1 U T4 DNA ligase for 4 h at room temperature and transformed competent XL1-Blue *Escherichia coli* cells (Stratagene) by the following incubation: 4°C for 30 min, 42°C for 90 s, and 4°C for 2 min in Falcon 2059 tube (Becton Dickinson Labware, Lincoln Park, NJ). Bacteria and DNA solutions were then diluted 10 times by LB broth medium and incubated for 1 h at 37°C. Ten percent of these bacterial solutions were spread out onto LB agar plates containing ampicillin (100 μg/ml), X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside), and IPTG (isopropylthio-β-D-galactoside); 50 μl of 20 mg/ml X-gal, and 10 μl of 10 mM IPTG were spread out per plate before plating transformed XL1 Blue cells. After incubating these plates at 37°C overnight, white-colored bacterial colonies were picked and the size of insert DNAs in the phagemid was monitored by PCR using a ScreenTest kit (Stratagene). *E. coli* cells harboring phagemids that contain the expected size of DNA insert were grown in 50 μg/ml ampicillin containing Super Broth (Quality Biological, Gaithersburg, MD) at 37°C overnight; phagemid DNA was isolated by Qiawell 8 System (Qiagen, Chatsworth, CA) according to the manufacturer's instructions and was sequenced by a method of cycle sequencing using M13 forward and reverse Dye Primer Prism Kit (Applied Biosystems, Foster City, CA) and an automated DNA sequencer (Applied Biosystems, model 373A). Each clone was sequenced in both orientations using M13 forward and reverse dye primers and confirmed both sequences were identical.

Sequence analysis

Sequence assembly and general sequence analysis were performed using the UWGCG program (19). Database search to identify similar sequences was performed by the NCBI BLAST program (20). Phylogenetic analyses were performed by the neighbor-joining method (21), and the protein sequence parsimony method (PROTPARS)[3] (22) using PHYLIP program (22). Nonsynonymous and synonymous nucleotide substitution rates were calculated by Nei and Gojobori's method (23). All sequences studied here were submitted to GenBank by the following accession numbers.

MhcFeca:
*DRB*0101*, U51480; *DRB*0102*, U51482; *DRB*0103*, U51483; *DRB*010401*, U51484; *DRB*0105*, U51485; *DRB*0106*, U51486; *DRB*0107*, U51487; *DRB*0108*, U51488; *DRB*0109*, U51489; *DRB*0110*, U51490; *DRB*0111*, U51491; *DRB*0112*, U51492; *DRB*0113*, U51493; *DRB*0114*, U51494; *DRB*0201*, U51496; *DRB*0202*, U51497; *DRB*0203*, U51498; *DRB*0204*, U51499; *DRB*020501*, U51500; *DRB*0206*, U51983; *DRB*0207*, U51501; *DRB*0208*, U51502; *DRB*0209*, U51503; *DRB*0210*, U51504; *DRB*0211*, U51505; *DRB*0212*, U51506; *DRB*0213*, U51507; *DRB*0214*, U51508 (exon 2 sequence of DRB*0214 cDNA clone); *DRB*0215*, U51509; *DRB*0216*, U51510; *DRB*0217*, U51511; *DRB*0218*, U51512; *DRB*0219*, U51513; *DRB*0301*, U51514; *DRB*0302*, U51515; *DRB*0303*, U51516; *DRB*0304*, U51517; *DRB*0305*, U51518; *DRB*0306*, U51519; *DRB*0307*, U51520; *DRB*0308*, U51521; *DRB*0309*, U51522; *DRB*0310*, U51523; *DRB*0311*, U51524; *DRB*0312*, U51525; *DRB*0313*, U51526; *DRB*040101*, U51527; *DRB*0402*, U51528; *DRB*0403*, U51529; *DRB*0404*, U51530; *DRB*0405*, U51531; *DRB*0501*, U51532; *DRB*0502*, U51984; *DRB*0503*, U51533; *DRB*0504*, U51534; *DRB*0505*, U51535; *DRB*0506*, U51536 (exon 2 sequence of DRB*0506 cDNA clone); *DRB*0507*, U51537 (exon 2 sequence of DRB*0507 cDNA clone); *DRB*0508*, U51538; *DRB*0509*, U51539; *DRB*0510*, U51540; *DRB*0511*, U51541; *DRB*0512*, U51542.
MhcLeti:
*DRB*0501*, U51543; *DRB*0502*, U51544; *DRB*0503*, U51545.
MhcMair:
*DRB*0401*, U51546; *DRB*0402*, U51547.
MhcOnge:
*DRB*0201*, U51548.
MhcFeca:
*DRB*0214*, U51574 (cDNA sequence); *DRB*0506*, U51575 (cDNA sequence); *DRB*0507*, U51573 (cDNA sequence).
MhcFeca:
*DRA*0101*, U51576 (cDNA sequence); *DRA*0102*, U51577 (cDNA sequence); *DRA*0103*, U51578 (cDNA sequence).

Results

Characterization of feline MHC DRA and DRB cDNA clones

Seven feline *DRA* and eight feline *DRB* cDNA clones were isolated by the secondary screenings of a spleen cell cDNA library from a domestic cat (Fca 021), using human *DRA* and *DRB1* cDNA as probes. Three unique *DRA* and three unique *DRB* sequences were obtained from analysis of these cDNA clones. Derived cDNA sequences showed high identity to human *DRA* or *DRB* sequences, respectively, by database search. For instance, feline *DRA* clones showed 89.4 to 90.0% nucleotide sequence identity to human *DRA* and less than 70% identity to human *DQA*, *DPA*, and *DNA*, while feline *DRB* clones showed 84% sequence identity to human *DRB*, 69.9 to 73.5% to human *DQB* and *DPB*, 66% to human *DOB* (Tables I and II). Three unique sequences for both *DRA* and *DRB* cDNA clones were isolated from a single cDNA library (Figs. 1 and 2), suggesting that feline MHC has a minimum of two loci for both *DRA* and *DRB* genes. Alignment of nucleotide and deduced amino acid sequences with those of human *DRA* and *DRB* genes revealed that mature feline *DRA* and *DRB* molecules consist of 229 amino acids (aa) and 237 (aa), respectively. The feline *DRA* molecule has a 25 aa leader sequence, 84 aa α1 and 107 aa α2 domains, a 23 aa transmembrane, and a 15 aa cytoplasmic domain (Fig. 1). The feline *DRB* molecule has a 29 aa leader sequence, 95 aa β1 and 103 aa β2 domains, a 23 aa transmembrane domain and a 16 aa cytoplasmic domain (Fig. 2).

Eighteen polymorphic amino acid residues were found among these three domestic cat *DRA* molecules, and none of these residues was located in the α1 domain, which is involved in the recognition of Ag and TCR (Fig. 3). In contrast, 33 of 35 polymorphic residues (94%) found in these three feline *DRB* sequences were located in the β1 domain, which is also involved in the recognition of Ag and TCR (Fig. 3). Nearly all of the polymorphic residues were located in its peptide-binding groove defined by an x-ray

[3] Abbreviations used in this paper: PROTPARS, protein sequence parsimony method; ARS, antigen recognition sites; NAR, nonantigen recognition sites; dS, synonymous nucleotide substitution rates; dN, nonsynonymous nucleotide substitution rates; aa, amino acid; LB, Luria-Bertani.

Table I. *The percentages of sequence identities among MHC class II α-chain coding sequences*[a]

	*Feca-DRA*0101*	*Feca-DRA*0102*	*Feca-DRA*0103*	*Bola-DRA*	*HLA-DRA*0101*	*HLA-DQA1*0101*	*HLA-DPA1*0301*	*HLA-DNA*
*Feca-DRA *0101*		98.5	96.7	88.2	89.7	63.3	68.4	65.9
*Feca-DRA*0102*	97.7		98.9	88.3	90.0	66.1	71.1	67.9
*Feca-DRA*0103*	94.5	98.6		87.5	89.4	63.9	69.3	66.9
Bola-DRA	84.9	84.5	83.7		85.7	64.3	68.9	67.0
*HLA-DRA*0101*	88.2	97.7	87.5	82.4		63.6	67.5	65.3
*HLA-DQA1*0101*	57.1	61.5	57.9	53.4	53.9		66.9	66.8
*HLA-DPA1*0301*	60.0	64.4	61.2	57.7	58.4	58.3		66.7
HLA-DNA	55.4	58.7	56.2	52.8	52.8	56.4	55.4	

[a] Values in upper triangle and lower triangle represent percentages of nucleotide sequence identities, and amino acid sequence identities, respectively.

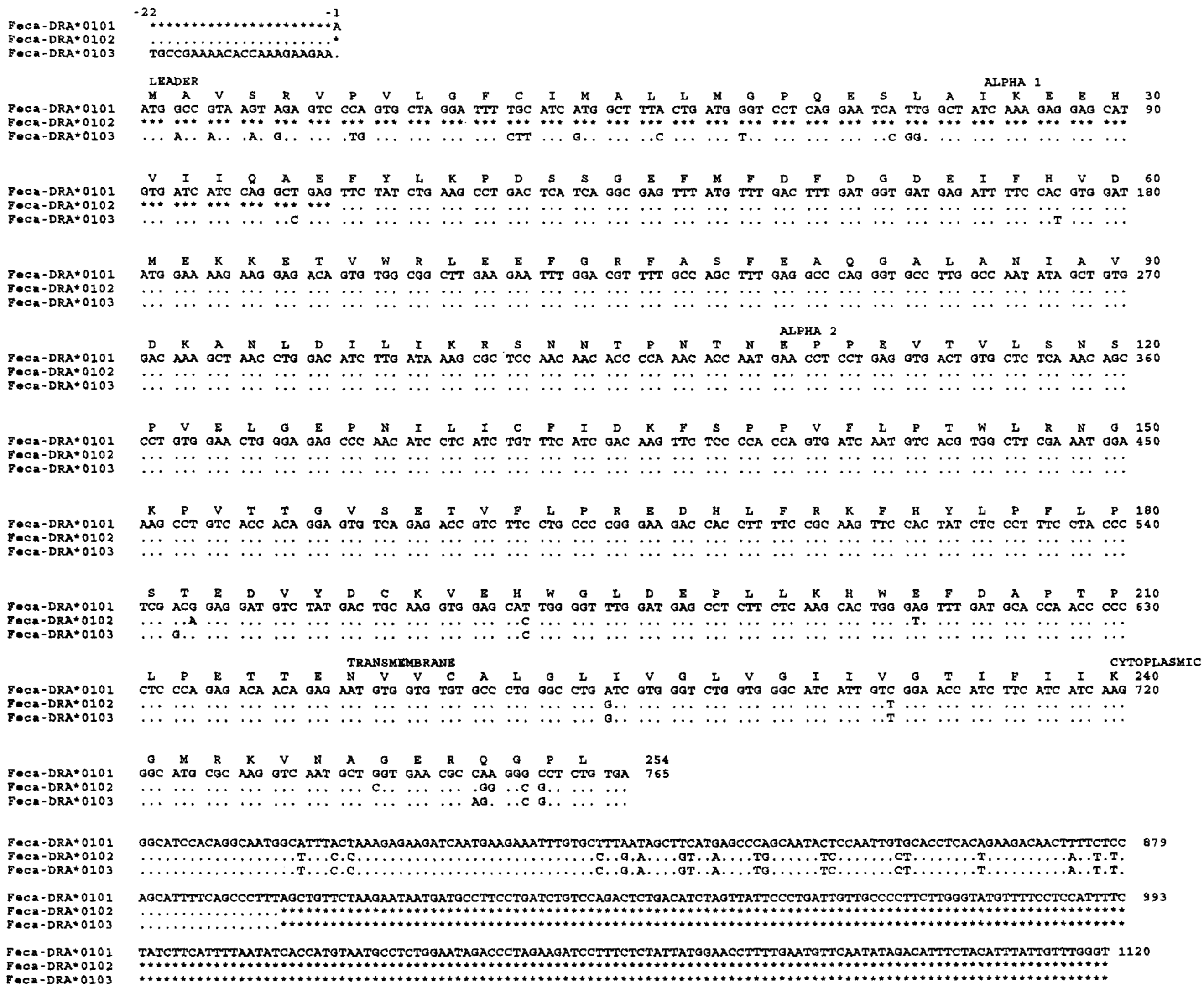


FIGURE 1. Nucleotide sequences of three MHC class II *DRA* cDNA clones of the domestic cat isolated from a single cDNA library. The complete nucleotide sequence and deduced amino acid sequence of *Feca-DRA*0101* allele is shown. Single letters and dots below the nucleotide sequence represent nucleotides that are, respectively, distinct from or identical to *DRA*0101*. Asterisks indicate missing sequences. Borders of each domain were assigned based upon sequence homology between *Feca-DRA*0101* and *HLA-DRA*0101*.

crystallographic model of *HLA-DR1* MHC class II molecules (24). Nearly half of these sites (14 of 33) shared identical polymorphic residues found in human class II β-chain molecule (24).

PCR-amplified exon 2 region of the MHC class II DRB gene in natural populations of the domestic cats

To investigate these highly polymorphic regions, which consist of Ag-binding sites, we used PCR (25) to amplify the second exon, which encodes the first extracellular domain of the *DRB* gene from 36 feral domestic cats in 11 geographic locales of eight countries (Table III). Sequence analyses of cloned *DRB* gene segments showed 61 distinct alleles by nucleotide sequences (Fig. 4); 59 alleles had a distinct amino acid sequence (Fig. 5).

In order to examine the relationship of these *DRB* sequences, we performed phylogenetic analysis for these feline *DRB* nucleotide sequences using the neighbor-joining method based on Kimura's

Table II. *The percentages of sequence identities among MHC class II β-chain coding sequences*[a]

	*Feca-DRB *0506*	*Feca-DRB *0507*	*Feca-DRB *0214*	*Dog MHC dw1*	*Bola-DRB3*	*HLA-DRB1 *0101*	*HLA-DQB1 *0501*	*HLA-DPB1 *01011*	*HLA-DOB*
*Feca-DRB*0506*		95.5	94.7	86.2	84.9	84.0	73.5	70.4	65.9
*Feca-DRB*0507*	93.3		93.4	86.5	84.8	84.1	73.2	69.9	65.9
*Feca-DRB*0214*	92.2	89.1		86.4	84.4	84.1	73.5	71.0	65.7
Dog MHCdw1	81.0	83.6	83.2		84.3	86.2	73.8	72.6	66.4
Bola-DRB3	80.5	80.9	79.1	79.7		85.3	71.3	70.4	64.8
*HLA-DRB1*0101*	77.9	79.4	78.3	81.9	77.5		71.8	71.8	64.5
*HLA-DQB1*0501*	63.7	64.5	64.4	68.3	59.1	62.2		73.7	67.1
*HLA-DPB1*01011*	57.9	56.8	59.2	64.3	57.9	60.6	63.4		63.4
HLA-DOB	51.9	52.3	53.5	57.8	51.9	53.8	56.6	51.2	

[a] Values in upper triangle and lower triangles represent percentages of nucleotide sequence identities, and amino acid sequence identities, respectively.

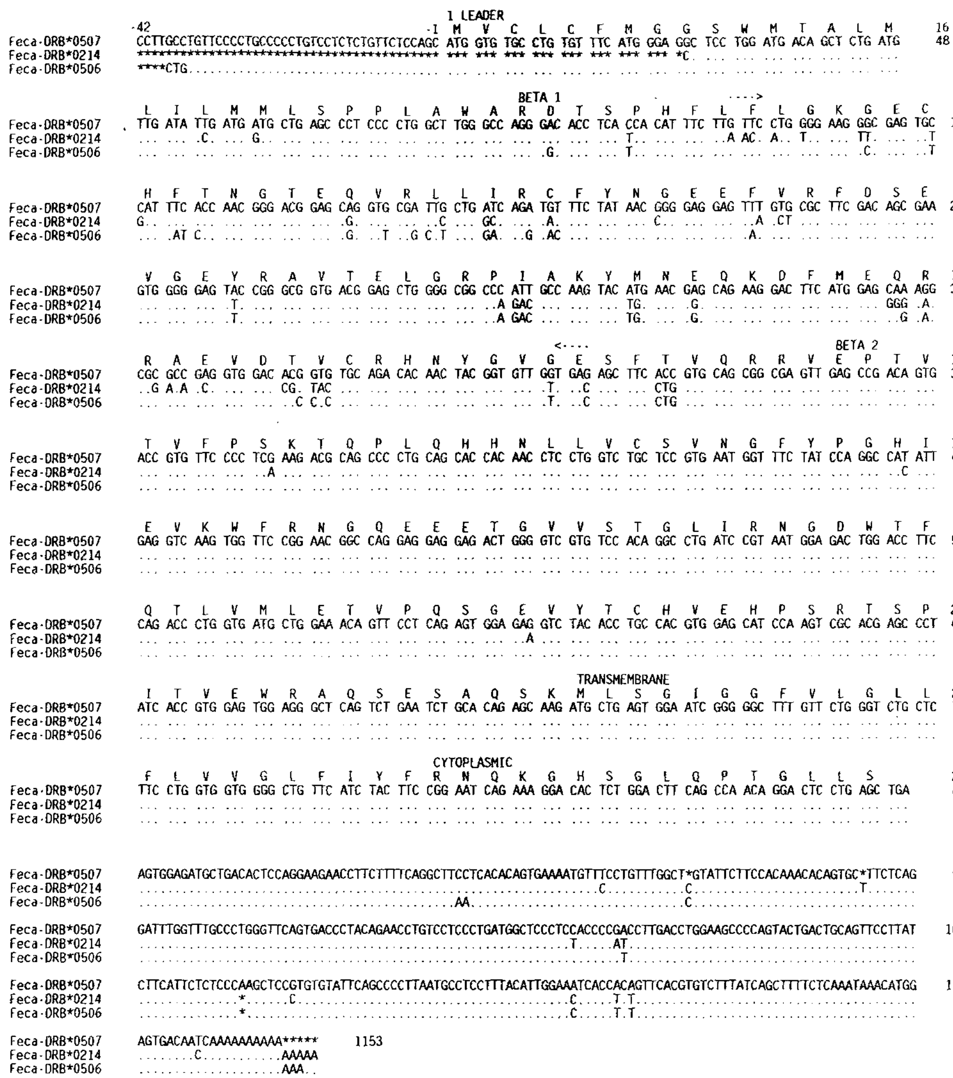


FIGURE 2. Nucleotide sequences of three MHC class II *DRB* cDNA clones of the domestic cat, isolated from a single cDNA library, FCA 021. Nucleotide and deduced amino acid sequences of the *Feca-DRB*0507* allele is shown. Single letters and dots below the sequence represent nucleotides that are, respectively, distinct from and identical to *DRB*0507.* Asterisks indicate missing sequences. Borders of each domain were assigned based on sequence alignment between *Feca-DRB*0507* and *HLA-DRB1*0101.* Arrows above the deduced amino acid sequence of β1 domain indicate PCR-amplified sequences excluding primer sequences.

two parameter distance (26). Six *DRB* sequences isolated from three nondomestic cat species—Geoffroy's cat (*O. geoffroyi*), tiger cat (*L. tigrina*), and Iriomote cat (*M. iriomotensis*)—were also dete rmined and analyzed with representative sequences from human *HLA-DRB 1, 3, 4, 5, 6, 7,* and *9* lineages, mouse *H-2Eb* alleles, and dog-*DRB* sequences as outgroups.

```
             LEADER             20    ALPHA 1         40                   60                   80                  100
Feca-DRA*0101 MAVSRVPVLGFCIMALLMGP QESLAIKEEHVIIQAEFYLK PDSSGEFMFDFDGDEIFHVD MEKKETVWRLEEFGRFASFE AQGALANIAVDKANLDILIK
Feca-DRA*0102 ******************** ****************.... .................... .................... ....................
Feca-DRA*0103 .TING.L....LIVAF..C. ...G................ .................... .................... ....................

                     ALPHA 2 120                  140                  160                  180                  200
Feca-DRA*0101 RSNNTPNTNEPPEVTVLSNS PVELGEPNILICFIDKFSPP VINVTWLRNGKPVTTGVSET VFLPREDHLFRKFHYLPFLP STEDVYDCKVEHWGLDEPLL
Feca-DRA*0102 .................... .................... .................... .................... ....................
Feca-DRA*0103 .................... .................... .................... .................... .A..................

                        TM                   CY
                       220                  240          254
Feca-DRA*0101 KHWEFDAPTPLPETTENVVC ALGLIVGLVGIIVGTIFIIK GMRKVNAGERQGPL
Feca-DRA*0102 ...V................ ....V............... .......R..R.A.
Feca-DRA*0103 .................... ....V............... ..........R.A.


             LEADER             20          BETA 1    40                   60                   80                  100
Feca-DRB*0507 MVCLCFMGGSWMTALMLILM MLSPPLAWARDTSPHFLFLG KGECHFTNGTEQVRLLIRCF YNGEEFVRFDSEVGEYRAVT ELGRPIAKYMNEQKDFMEQR
Feca-DRB*0214 *********.........S. V.............S..TMW .F..D.....R..F.A.Y.. ..R..LA........F.... .....D...W.G......GK
Feca-DRB*0506 .................... ...........G..S..... .A...YP...R....D.Y.. .....Y.........F.... .....D...W.G.......K

                                120      BETA 2       140                  160                  180                  200
Feca-DRB*0507 RAEVDTVCRHNYGVGESFTV QRRVEPTVTVFPSKTQPLQH HNLLVCSVNGFYPGHIEVKW FRNGQEEETGVVSTGLIRNG DWTFQTLVMLETVPQSGEVY
Feca-DRB*0214 .TA..RY.......VD..L. .................... .................... .................... ....................
Feca-DRB*0506 ......L.......VD..L. .................... .................... .................... ....................

                                220          TM                      CY      260
Feca-DRB*0507 TCHVEHPSRTSPITVEWRAQ SESAQSKMLSGIGGFVLGLL FLVVGLFIYFRNQKGHSGLQ PTGLLS
Feca-DRB*0214 .................... .................... .................... ......
Feca-DRB*0506 .................... .................... .................... ......
```

FIGURE 3. Aligned amino acid sequences of MHC class II *DRA* and *DRB* allelic sequences of the domestic cat, FCA 021. Amino acid sequences of *DRA*0101* and *DRB*0507* alleles are shown. Single letters and dots represent amino acid residues that are, respectively, distinct from and identical to the reference sequence. Asterisks indicate missing sequences.

The phylogenetic analysis (Fig. 6) revealed the following associations: 1) All feline sequences, both domestic and exotic species, are monophyletic (supported by a bootstrap score of 100%) and clearly divergent from human, mouse, and dog outgroup species. 2) The dog *DRB* sequences are the closest sequences to cat *DRB* (bootstrap for association equals 89%) as might be expected, since felids and canids are families of a single mammal order, *Carnivora*. 3) Five distinct clusters or allele lineages were resolved albeit with only modest bootstrap support and also confirmed by the PROTPARS method (data not shown); these groups are designated *Feca DRB*1* to *DRB*5*. 4) Allele transcripts from *DRB*1* to *DRB*5* lineages are distributed throughout the world (Table III). 5) Limited *DRB* alleles derived from three nondomestic species are monophyletic for the species and align with one of the five *Feca DRB* lineages. In every case, the association derives from a deep ancestral node suggesting considerable mutation divergence of the *DRB* gene in domestic and nondomestic cats since divergence from their common ancestor. The species associations were: Geoffroy's cat (*Onge-DRB*2*), Iriomote cat (*Mair-DRB*4*), and tiger cat (*Leti-DRB*5*). These phylogenetic results suggest that the DRB allelic lineage was generated prior to the divergence of modern feline species from their ancestors, but subsequent to the split of Felidae from Canidae and noncarnivore species.

Based upon the phylogenetic assortment, we designate the sequences as *MhcFeca-DRB*0101* to *MhcFeca-DRB*0512* and used the simplified name, *Feca-DRB*0101* to *Feca-DRB*0512* in this paper, based on the proposal of nomenclature convention (27). The feline *DRB* sequences are likely alleles encoded by two or three *DRB* genes in domestic cats. Approximately 80% of domestic cats, in which more than four *DRB* clones were examined, contained three or more *DRB* allelic sequences and five cats had five or six *DRB* sequences (Table III). In five cases, we found that three sequences identified from a single cat fell in a same lineage. Of these, three cases showed three nearly identical sequences (0105/0106/0107 in FCA183, 0507/0508/0509 in FCA017, and 0208/0209/0211 in FCA057), suggesting that each phylogenetic lineage is not restricted to a single locus, but recent gene duplication events subsequent to the generation of major phylogenetic lineages of feline *DRB* genes. The remaining two cases contained two closely related sequences plus one chimeric sequence. In one case (0104/0114/0115 in FCA177), 0114 sequence is a chimeric sequence between 0101 and 0104, indicating that two independent events—gene duplication plus interallelic (lineage) recombination—are possible mechanisms to explain these sequences. In the second case (0302/0305/0306), 0302 sequence is a chimera between 0303 and the last 30 bases of 0213, suggesting recombination between two lineages, in addition to a gene duplication event, could occur for the generation of this type of *DRB* polymorphism in feline MHC (Table III and Fig. 4). We also designated three *DRB* lineages from three exotic cat species as follows: *MhcOnge-DRB*2* for Geoffroy's cat (*O. geoffroyi*), *MhcMair-DRB*4* for Iriomote cat (*M. iriomotensis*), *MhcLeti-DRB*5* for tiger cat (*L. tigrina*).

Positions of polymorphic sites in deduced amino acid sequences of feline DRB genes

In order to examine the nature of polymorphism found in the PCR-amplified exon 2 coding region of feline *DRB* genes, the positions of polymorphic amino acid residues in 61 cat *DRB* alleles were compared with those of 111 human *DRB* exon 2 sequences (provided by Dr. Colm O'hUigin, Max Planck Institute, Tübingen, Germany). Patterns of polymorphic sites were compared with the positions of Ag recognition sites (ARS), designated by ARS definition based on x-ray crystallography of *HLA-DR1* class II molecules (24) (Fig. 7). For 111 human *HLA-DRB* alleles, 124 polymorphic amino acid residues were found in a 95 aa segment (95 sites) of the first extracellular domain, (1.30 polymorphic residues/site) and of these 63 polymorphic residues are located on defined (22 sites) ARS, respectively (Fig. 7). These results indicated significant accumulation of polymorphic sites in ARS of human *HLA-DRB* molecules (2.86 polymorphic residues/site in

Table III. *DRB alleles of domestic cats*[a]

Cat Code	Location	No. of *DRB* Clones Sequenced	No. of *DRB* Alleles Found	*DRB* Alleles Present				
				*DRB*1*	*DRB*2*	*DRB*3*	*DRB*4*	*DRB*5*
FCA168	AUS	1	1			0304		
FCA169	AUS	4	3		0201, 0204	0308		
FCA170	AUS	1	1	0107				
FCA171	AUS	3	2	0102				0511
FCA172	AUS	3	2			0301, 0304		
FCA174	AUS	2	1	0111				
FCA175	AUS	3	2	0108	0206			
FCA177	AUS	4	3	0103, 0113, 0114				
FCA178	THAI	3	3	0104		0310	0403	
FCA179	THAI	4	3		0213			0501, 0503
FCA180	THAI	1	1					0502
FCA181	THAI	4	3	0107	0202	0304		
FCA182	THAI	5	5		0210, 0216	0304, 0307		0501
FCA183	THAI	8	4	0104, 0105, 0106	0207			
FCA184	THAI	2	2			0304, 0307		
FCA144	RUS STP	4	2	0107	0201			
FCA145	RUS STP	1	1		0212			
FCA120	IND	3	2	0107		0304		
FCA150	CAM	5	5	0103, 0107	0215		040101, 0402	
FCA151	NCG	5	4		0201	0302, 0305, 0306		
FCA153	ARG BA	3	2	0102, 0107		0304		
FCA154	ARG BA	3	3	0107				0504, 0512
FCA155	ARG MZ	2	2	0101, 0107				
FCA156	ARG CO	4	3	0109, 0110				0505
FCAOT3	UK	3	3	0103, 0107			0405	
FCATA2	UK	2	2			0303, 0304		
FCACW2	UK	2	2	0112	0219			
FCA011	USA MD	4	2		0203		0403	
FCA017	USA MD	7	5	0107		0312		050701, 0508, 0509
FCA021	USA MD	8	3		0214			0506, 050702
FCA039	USA MD	4	2		0207	0311		
FCA042	USA MD	8	1				040101	
FCA057	USA MD	11	6		0208, 0209, 0211	0311	040102, 0404	
FCA062	USA MD	4	4		020501, 0209	0303, 0304		
FCAC114	USA MD	3	2		020502, 0218			
FCAPA107	USA PA	8	5		0209	0310, 0311		050702, 0510
FCAPA116	USA PA	2	2			0310, 0313		

[a] Geographic locations for sample collections were indicated as abbreviated letters as follows: ARG BA, Argentina Buenos Aires; ARG CO, Argentina Cordoba; ARG MZ, Argentina Mendoza; AUS, Australia; CAM, central America (exact location was not known); IND, India; NCG, Nicaragua; RUS STP, Russia, St. Petersburg; THAI, Thailand, UK, United Kingdom; USA MD, United States of America, Maryland; USA PA, United States of America, Pennsylvania.

defined ARS). Sixty-three feline *DRB* alleles display 115 polymorphic residues in a 78 aa segment of first extracellular domain-coding region (1.47 polymorphic residues/site). Of these, 58 were in the 22 positions of the defined ARS (2.6 residues/site for the defined ARS). These results indicated that distributions of polymorphic residues in cat *DRB* is rather similar to human *DR* molecules, except that Feca *DRB* alleles have more polymorphic residues than human *DRB* molecules at the last three ARS (positions 78, 81, and 86).

Nucleotide substitution patterns of ARS and non-ARS of feline DRB molecules

The pattern of nucleotide substitution in feline *DRB* sequence at ARS and non-ARS sites defined by the x-ray crystallographic model (28–30) were examined. We calculated synonymous (dS) and nonsynonymous (dN) nucleotide substitution rates for the ARS and non-ARS in all pairwise comparisons of 49 *DRB* sequences (13, 14, 30) using Jukes and Cantor's genetic distance (31). Average dS and dN estimates for each feline *DRB* sequence comparison are presented in Figure 8. With a few exceptions, dN values were greater than dS values for ARS, while the reverse is true for non-ARS sites (Fig. 8). These results are parallel to the analyses of MHC class I genes (13, 14, 30), although the ratios of dN and dS values in the ARS of class I molecules are much higher than those of class II *DRB* molecules (dN = 15.2, dS = 7.2 for domestic cat class I molecules' ARS (13). This was caused by lower average dN value in ARS sites of feline *DRB* molecules compared with dN values in ARS sites of human *DRB* and mouse *Eb* molecules (18.5 for feline *DRB*, and 45.7 and 41.5 for human *DRB* and mouse *Eb* molecules, respectively) since the dS value in the ARS of feline *DRB* molecules is equivalent to values of two other species (13.3 for feline *DRB*, 15.0 for human *DRB*, and 11.6 for mouse *Eb* molecules) (32 and this study).

Discussion

Sequence analyses of domestic cat MHC class II *DRA* and *DRB* genes showed remarkable similarities to the human HLA class II *DR* system in the quantity and quality of polymorphism for *DRA* and *DRB* class II genes. In human HLA, class II *DRB* genes are highly polymorphic with some 126 *DRB* alleles identified to date (Table IV). In contrast,

```
                                      20                   40                   60                   80                  100                  120
Feca DRB*0101   GCTCCTGTGGAAGAGCGAGT GCCATTTCACCAACGGGACG GAGCAGGTGCGACTCCTGGA GAGACACTTCTATAACGGGG AGGAGTTTGTGCGCTTCGAC AACGAAGTGGGGAGTTCCG
         0102   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
         0103   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
         0104   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
         0105   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G.A................
         0106   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
         0107   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
         0108   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
         0109   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
       / 0110   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G....A.............
         0111   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
         0112   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .G..................
         0113   AAATA...T....GC..... .................... ............T......T C...TGT............. ......A............. .GT.................
         0114   AAATA............... .................... ............T......T C...TGT............. ......A............. .G..................
Feca DRB*0201   AAC.A........TT..... .T.................. ....G......GTA.....T C...TTT.........C... .......A.CT......... .G..................
         0202   AAC.A........TT..... .T.................. ....G......GTA.....T C...TTT.........C... .......A.CT......... .G..................
         0203   AAC.A........TT..... .T...ATC............ ....G.......TA.....T C...TTT.........C... .......A.CT......... ....................
         0204   AAC.A........TT..... .T...ATC............ ....G.......TA.....T C...T.T.........C... .......A.CT......... .G..................
       020501   AA..A..G.....GC..... .................... ....G.......T......C C...T.T.........C... .......A.CT......... .G..................
       020502   AA..A..G.....GC..... .................... ....G.......T......C C...T.T.........C... .......A.CT......... .G..................
         0206   AA..A..G.....GC..... .................... ....G.......T......C C...T.T.........C... .......A.CT......... .G..................
         0207   AA..A..G.....GC..... .................... ....G.....A.T......C C...T.T.........C... .......A.CT......... .G..................
         0208   AA..A..G.....GC..... C................... ....G.....A.T......C C...T.T.........C... .......A.CT......... .G..................
         0209   AA..A..G.....GC..... .................... ....G.......T......C C...T.T.........C... .......A.CT......... .G..................
         0210   AA..A..G.....GC..... .................... ....G.......T......C C...T.T.........C... .......A.CT......... GG..................
         0211   AA..A..G.....GC..... .................... ....G.....A.T......C C...T.T.........C... .......A.CT......... .G..................
         0212   AG..A..G.....GC..... .T.................. ............T......C C...T.T.........C... .......A.CT......... .G..................
         0213   AAC.A........TT..... .TG................. ....G.......T......C C...T.T.........C... .......A.CT......... .G..................
         0214   AAC.A........TT..... .TG................. ....G.......T......C C...T.T.........C... .......A.CT......... .G..................
         0215   AAC.A........TT..... .T.................. ....G.......T......C C...T.T.........C... .......A.CT......... .G..................
         0216   AAC.A........TT..... .T.................. ....G.......T......C C...T.T.C.......C... .......A.CT......... .G..................
         0217   AAC.A........TT..... .T.................. ....G.......T......C C...T.T.........C... .......A.CT......... .G..................
         0218   AAC.A........TT..... .T.................. ....G......GTA.....T C...TTT.........T... .......A.CT......... .G..............C...
         0219   AAC.A........TT..... .T...ATC............ ....G.......TA.....T C...TTT.........C... .......A.CT......... ....................
Onge DRB*0201   .T.....G......C..... .T.................. ....G.......T....... C...T.T.........C... .......A.CT......... .G..........A...A...
Feca DRB*0301   AAATA........GCG.... ...................A ....G......GTA...... ........ATC......... .....AACT........... .G..............A...
         0302   AAATA........GCG.... ...................A ....G......GTA...... ........ATC......... .....AACC........... .G..............A...
         0303   AAATA........GCG.... ...................A ....G......GTA...... ........ATC......... .....AACT........... .G..............A...
         0304   AAATA........GCG.... ...................A ....G......GTA...... ........ATC......... .....AACT........... .G..............A...
         0305   AAATA........GCG.... ...................A ....G......GTA...... ........GTC......... .....AACT........... .G..............A...
         0306   AAATA........GCG.... ...................A ....G......GTA...... ........ATC......... .....AACT........... .G..............A...
         0307   AAATA........GCG.... ...................A ....G......GTA...... ........ATC......... .....AACT........... .G..............A...
         0308   AAATA........GCG.... ...................A ....G......GTA...... ........ATC......... .....AACT........... .G..............A...
         0309   AAC.A........TT..... .T.................A ....G......GTA...... ........ATC......... .....AACT........... .G..............A...
         0310   AAATA........GCG.... .................... ....G......GTA....CG .......G..........T. ......A.........T... .G..................
         0311   AAATA........GCG.... .................... ....G...TT.G........ .................... ......G.........T... .G..................
         0312   AAATA........GCG.... ...G................ ....G...TT.G........ .................... ......G.........T... .G..................
         0313   AAATA........GCG.... .................... ....G...TT.G........ .................... ......G.........T... .G..................
Feca DRB*040101 .T.....G......C..... .................... ....G...T..G........ C...T............... ......A............. .G..................
       040102   .T.....G......C..... .................... ....G...T..G........ C...T............... ......A............. .G..................
         0402   .T..A..G......C..... .................... ....G...T..G........ C...T............... ......A............. .G.A................
         0403   .................... .................... ....G...T..G..T..... C...T............... ......A............. .G..................
         0404   ...............T.... .................... ...AG...T..G..T..... C...T............... ......A......C...... .G..................
         0405   .................... .................... ....G...T..G..T..... CG..T............... .....CA............. .G..................
Mair DRB*0401   AAATA......G.TC..... .................... ....G............... C...T............... .....AC............. .G.A................
         0402   AAATA......G.TC..... .................... ....G............... C...T............... .....AC............. .G..................
Feca DRB*0501   .................... .................... ....G.......T....... C...TGT............. .................... ................A...
         0502   .........•.......... .................... ....G.......T....... C...TGT............. .................... .........T......A...
         0503   CTC.TGTG.A.......... .................... ....G.......T....... C...TGT............. .................... ................A...
         0504   .T.....G.....GC..... .T...ATC............ ....G...T..G..T..... C..GT............... ......A............. .G..................
         0505   .T.....G.....GC..... .T...ATC............ ....G...T..G..T..... C..GT............... ......A............. .G..................
         0506   .T.....G.....GC..... .T...ATC............ ....G...T..G..T..... C..GT............... ......A............. .G..................
       050701   .T.....G.....G...... .................... ............T.G...AT C...TGT............. ..........C......... .G..............A...
       050702   .T.....G.....G...... .................... ............T.G...AT C...TGT............. .................... .G..............A...
         0508   .T.....G.....G...... .................... ............T.G...AT C...TGT............. .................... .G..............A...
         0509   .T.....G.....GC..... .................... ........C...T.G...AT C...TGT............. .................... .G..............A...
         0510   .T.....G.....G...... .................... ............T.G...AT C...TGT............. .................... .G..............A...
         0511   .T.....G.....G...... .................... ............T.G...AT C...TGT............. .................... .G..............A...
         0512   .................... .................... .................... .................... ...............C.... ....................
Leti DRB*0501   .T.....G.....GC..... .T...ATC............ ....G.......T.A..... C...T............... ......A............. .G..................
         0502   .T.....G.....G...... .T...ATC............ ....G.......T.A..... C...T............... ......A............. .G..................
         0503   .T.T...G.....G...... .T...ATC............ ....G.......T.A...C. C...TG.............. .................... .G..................
```

FIGURE 4. A complete list of nucleotide sequences of β1 domain coding region of feline MHC class II *DRB* genes. Single letters and dots represent nucleotides that are, respectively, distinct from and identical to *DRB*0101*. The allelic numbers for domestic cat and exotic cats were assigned based upon five major lineages of *DRB* alleles found by phylogenetic analysis (Fig. 6) and sequence alignments of amino acid sequences by PILEUP program of UWGCG (Fig. 5). MHC abbreviations used for species were *Feca, Felis catus* (domestic cat), *Onge, Oncifelis geoffroyi* (Geoffroy's cat), *Mair, Mayailurus iriomotensis* (Iriomote cat), *Leti, Leopardus tigrina* (tiger cat).

the *DRA* gene, which encodes the other half of a heterodimeric *DR* class II molecule, has only two alleles in human populations (33). The domestic cat MHC encodes at least 61 *DRB* alleles in worldwide populations of the domestic cat, while we resolved only one *DRA* allele by the sequences in exon 2, although nucleotide substitutions were found in other regions (Fig. 1). Similarly, a majority of mammalian MHC studied so far maintained a number of *DRB* alleles (Table IV), while *DRA* gene is monomorphic (34).

A comparative analysis of the positions of polymorphic residues in the first extracellular domain of the *DRB* molecule in humans and domestic cats showed the extraordinary similarity of positions of polymorphic residues facing defined ARS (Fig. 7). Furthermore, examination of the patterns of nucleotide substitutions in ARS, and other regions in exon 2 of these feline *DRB* genes indicates selection pressures observed in feline class II genes, which include positive selections for amino acid substitutions in the Ag-binding site, but negative selection pressure against codon changes in the framework and NAR of *DRB* molecules. This evidence seems to suggest strong functional constraints for the patterns of polymorphism in Ag-binding sites of *DRA* and *DRB* molecules during long divergence time between humans and the domestic cat.

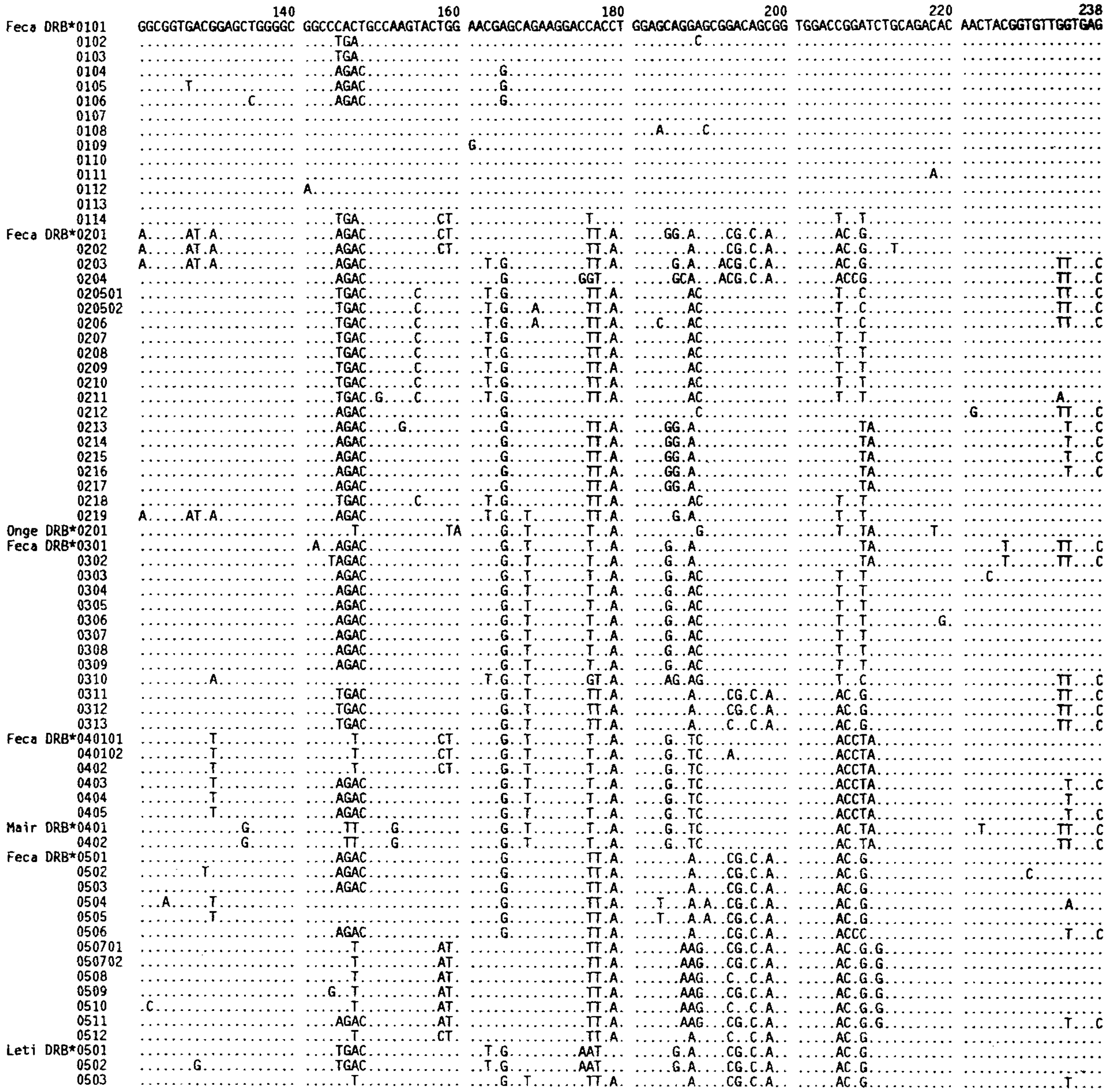


FIGURE 4. *(continued)*

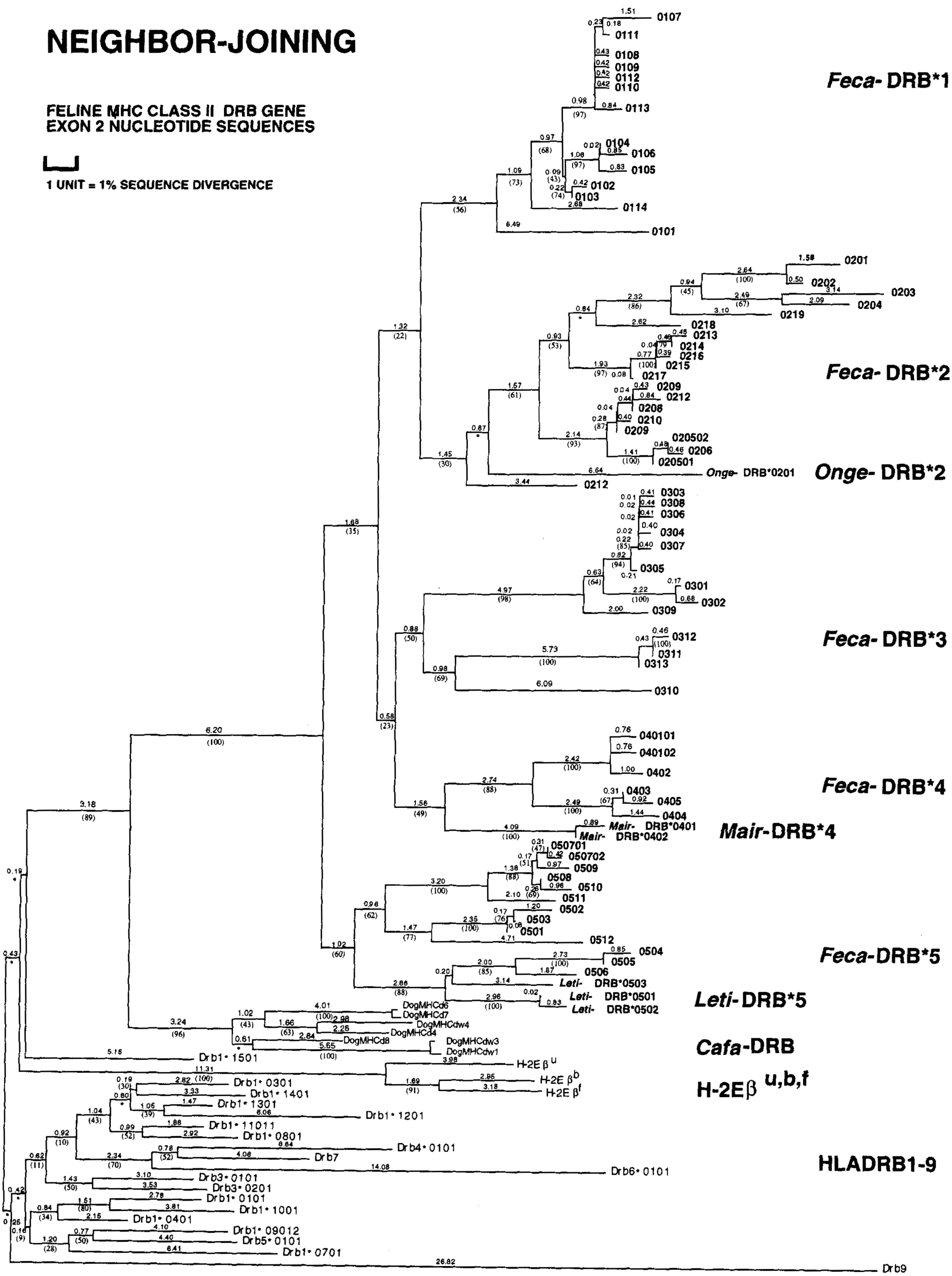
The occurrence of three distinct cDNA clones for both *DRA* and *DRB* genes from a single spleen cell cDNA library plus the occurrence of five or six *DRB* sequences in five cats (Table III) suggests that feline MHC contains at least two *DRA* loci and three *DRB* loci. Phylogenetic analysis for feline *DRB* sequences revealed five lineages of *DRB* allelic sequences that originated after the divergence of Felidae from carnivore families (e.g., canids), but before the more recent emergence of three major taxonomic groups of Felidae—ocelot, domestic cat, and pantherine lineages (6, 7). Identification of three sequences within a single phylogenetic lineage identified from a single cat in five cases suggests two possible events, 1) recent gene duplication, and 2) segmental exchanges between two lineages. Of these two possible events, recent gene duplication events of *DRB* genes is the most likely explanation, since apparent evidences of segmental exchanges between two lineages were rare. Only *Feca DRB*0302* in these five cases has chimeric structure between *Feca*0303* and the last 30 bases of 0213. The close association of *DRB* alleles of three nondomestic species (Geoffroy's cat, Iriomote cat, and tiger cat) with domestic cat *DRB* lineages suggests persistence of MHC allelic lineages through multiple speciation events (a trans-species mode of evolution) during the Felidae radiation since the last 10 to 15 million years.

Apparent mosaic sequences observed in feline MHC class I genes, which suggested inter- and intralocus segmental exchanges as a primary factor for the evolution of class I genes, were rarely seen in feline *DRB* exon 2 sequences, suggesting different modes of evolution operate diversification of feline MHC class I and class II *DRB* genes. Nonetheless, the trans-species pattern of allele divergence, the abundant allelic diversity, and the positive selection in favor of divergence observed in the ARS are consistent with an important role for the *DR* loci in immune response to pathogenic agents in ancient and modern species of felids.

```
                                     20                   40                   60                  79
Feca DRB*0101  LLWKSECHFTNGTEQVRLLE RHFYNGEEFVRFDNEVGEFR AVTELGRPTAKYWNEQKDHL EQERTAVDRICRHNYGVGE
         0102  NML.A............F.V .C......Y...S...... ........D........... ..A................
         0103  NML.A............F.V .C......Y...S...... ........D........... ...................
         0104  NML.A............F.V .C......Y...S...... ........D.....G..... ...................
         0105  NML.A............F.V .C......Y...S...... ........D.....G..... ...................
         0106  NML.A............F.V .C......Y...S...... ....P...D.....G..... ...................
         0107  NML.A............F.V .C......Y...S...... .................... ...................
         0108  NML.A............F.V .C......Y...S...... .................... ..D................
         0109  NML.A............F.V .C......Y...S...... ............D....... ...................
         0110  NML.A............F.V .C......Y...S.M.... .................... ...................
         0111  NML.A............F.V .C......Y...S...... .................... ............N......
         0112  NML.A............F.V .C......Y...S...... ......Q............. ...................
         0113  NML.A............F.V .C......Y...S...... .................... ...................
         0114  NM...............F.V .C......Y...S...... ........D...L.....Y. ........WF.........

Feca DRB*0201  TM..F.........R..Y.V .F...R..LA...S...... ..S.....D...L.....FM .GK.AE..TV.........
         0202  TM..F.........R..Y.V .F...R..LA...S...... ..S.....D...L.....FM ..K.AE..TVF........
         0203  TM..F...YP....R..Y.V .F...R..LA.......... ..S.....D.....G...FM .RKHAE..TV.......FD
         0204  TM..F...YP....R..Y.V .Y...R..LA...S...... ........D.....G..EV. .RKHAE..TV.......FD
         0205  IMG.A.........R..F.A .Y...R..LA...S...... ........D..H..G...FM ..T.....WL.......FD
         0206  IMG.A.........R..F.A .Y...R..LA...S...... ........D..H..G...FM D.T.....WL.......FD
         0207  IMG.A.........R.QF.A .Y...R..LA...S...... ........D..H..G...FM ..T.....WF.........
         0208  IMG.A.S.......R.QF.A .Y...R..LA...S...... ........D..H..G...FM ..T.....WF.........
         0209  IMG.A.........R..F.A .Y...R..LA...S...... ........D..H..G...FM ..T.....WF.........
         0210  IMG.A.........R..F.A .Y...R..LA...G...... ........D..H..G...FM ..T.....WF.........
         0211  IMG.A.........R.QF.A .Y...R..LA...S...... ........DG.H..G...FM ..T.....WF.......S.
         0212  VMG.A............F.A .Y...R..LA...S...... ........D.....G..... ..A..........S...FD
         0213  TM..F..D......R..F.A .Y...R..LA...S...... ........D.R...G...FM .GK......Y.......VD
         0214  TM..F..D......R..F.A .Y...R..LA...S...... ........D.....G...FM .GK......Y.......VD
         0215  TM..F.........R..F.A .Y...R..LA...S...... ........D.....G...FM .GK......Y.......VD
         0216  TM..F.........R..F.A .YS..R..LA...S...... ........D.....G...FM .GK......Y.......VD
         0217  TM..F.........R..F.A .Y...R..LA...S...... ........D.....G...FM .GK......Y.........
         0218  TM..F.........R..Y.V .F...W..LA...S....S. ........D..H..G...FM ..T.....WF.........
         0219  TM..F...YP....R..Y.V .F...R..LA.......... ..S.....D.....GL..FM .RK.....WF.........
Onge DRB*0201  F.G.T.........R..F.D .Y...R..LA...S...KY. ........I...L.GL..YM ..G.....WY..Y......

Feca DRB*0301  NM..A.........R..Y.. ..YH....NL...S....Y. ........D.....GL..YM .EK......Y.......FD
         0302  NM..A.........R..Y.. ..YH....NL...S....Y. .......LD.....GL..YM .EK......Y.......FD
         0303  NM..A.........R..Y.. ..YH....NL...S....Y. ........D.....GL..YM .ET.....WF....H....
         0304  NM..A.........R..Y.. ..YH....NL...S....Y. ........D.....GL..YM .ET.....WF.........
         0305  NM..A.........R..Y.. ..CH....NL...S....Y. ........D.....GL..YM .ET.....WF.........
         0306  NM..A.........R..Y.. ..YH....NL...S....Y. ........D.....GL..YM .ET.....WF..R......
         0307  NM..A.........R..Y.. ..YH....NL...S....Y. ........D.....GL..YM .ET.....WF.........
         0308  NM..A.........R..Y.. ..YH....NL...S....Y. ........D.....GL..YM .ET.....WF.........
         0309  TM..F.........R..Y.. ..YH....NL...S....Y. ........D.....GL..YM .ET.....WF.........
         0310  NM..A.........R..Y.R ..V.....Y...S...... ..............GL..VM .RR.....WL.......FD
         0311  NM..A.........R.W... ........C...S...... ........D.....GL..FM ..K.AE..TV.......FD
         0312  NM..A..R......R.W... ........C...S...... ........D.....GL..FM ..K.AE..TV.......FD
         0313  NM..A.........R.W... ........C...S...... ........D.....GL..FM ..K..E..TV.......FD

Feca DRB*0401  F.G.T.........R....D .Y......Y...S...... ........I...L.GL..YM .ES.....TY.........
         0402  FMG.T.........R....D .Y......Y...SK..... ........I...L.GL..YM .ES.....TY.........
         0403  ..............R....D .Y......Y...S...... ........D.....GL..YM .ES.....TY.......VD
         0404  ..............R....D .Y......Y..L.S..... ........D.....GL..YM .ES.....TY.......V.
         0405  ..............R....D GY......H...S...... ........D.....GL..YM .ES.....TY.......VD
Mair DRB*0401  NM.R..........R....D .Y......Y...SK..... ....V...F.E...GL..YM .ES.....TY.......FD
         0402  NM.R..........R....D .Y......Y...S...... ....V...F.E...GL..YM .ES.....TY.......FD

Feca DRB*0501  ..............R..F.D .C.................Y. ........D.....G...FM ..K.AE..TV.........
         0502  ..............R..F.D .C...............W.Y. ..M.....D.....G...FM ..K.AE..TV.........
         0503  SCG...........R..F.D .C.................Y. ........D.....G...FM ..K.AE..TV.........
         0504  F.G.A...YP....R....D .Y......Y...S...... ..............G...FM D.K.AE..TV.......D.
         0505  F.G.A...YP....R....D .Y......Y...S...... ..............G...FM D.K.AE..TV.........
         0506  F.G.A...YP....R....D .Y......Y...S...... ........D.....G...FM ..K.AE..TL.......VD
         0507  F.G.G..............I .C...........S....Y. ........I...M.....FM ..R.AE..TV.........
         0508  F.G.G..............I .C...........S....Y. ........I...M.....FM ..R..E..TV.........
         0509  F.G.A..............I .C...........S....Y. .......RI...M.....FM ..R.AE..TV.........
         0510  F.G.G..............I .C...........S....Y. P.......I...M.....FM ..R..E..TV.........
         0511  F.G.G..............I .C...........S....Y. ........D...M.....FM ..R.AE..TV.......VD
         0512  .................... ..........S......... ........I...L.....FM ..K..E..TV.........
Leti DRB*0501  F.G.A...YP....R....D .Y......Y...S...... ........D.....G..EI. .RK.AE..TV.........
         0502  F.G.G...YP....R....D .Y......Y...S...... ..A.....D.....G..EI. .RK.AE..TV.........
         0503  F.G.G...YP....R....H .C...........S...... ........I.....GL..FM ..K.AE..TV.......V.
```

FIGURE 5. Feline MHC class II *DRB* allele. Amino acid sequences of PCR amplified first extracellular domain coding region of *DRB* genes. The position 1 of this figure corresponds to position 9 of β1 domain (See Fig. 7).

FIGURE 6. Neighbor-joining tree using 238-bp nucleotide sequences of β1 domain coding region in MHC class II *DRB* genes based on Kimura's two-parameter distance. Phenetic distance branch length (percent sequence divergence) is indicated above each branch, numbers in parentheses are bootstrap percentages (out of 100 iterations) in support of each node. MHC abbreviations used for species are indicated as follows: *Feca, Felis catus* (domestic cat), *Onge, Oncifelis geoffroyi* (Geoffroy's cat), *Mair, Mayailurus iriomotensis* (Iriomote cat), *Leti, Leopardus tigrina* (tiger cat), *Cafa, Canis familiaris* (domestic dog). *H-2E β* and *HLADRB* are used for murine and human class II β-chain sequences, respectively.


NEIGHBOR-JOINING
FELINE MHC CLASS II DRB GENE
EXON 2 NUCLEOTIDE SEQUENCES
1 UNIT = 1% SEQUENCE DIVERGENCE
0107
0111
0108
0109
0112
0110
0113
0104
0106
0105
0102
0103
0114
0101
Feca- DRB*1
0201
0202
0203
0204
0219
0218
0213
0214
0216
0215
0217
0209
0212
0208
0210
0209
020502
0206
020501
Onge- DRB*0201
0212
Feca- DRB*2
Onge- DRB*2
0303
0308
0306
0304
0307
0305
0301
0302
0309
0312
0311
0313
0310
Feca- DRB*3
040101
040102
0402
0403
0405
0404
Mair- DRB*0401
Mair- DRB*0402
Feca- DRB*4
Mair-DRB*4
050701
050702
0509
0508
0510
0511
0502
0503
0501
0512
0504
0505
0506
Leti- DRB*0503
Leti- DRB*0501
Leti- DRB*0502
Feca-DRB*5
Leti-DRB*5
DogMHCd6
DogMHCd7
DogMHCdw4
DogMHCd4
DogMHCd8
DogMHCdw3
DogMHCdw1
Cafa-DRB
Drb1*1501
H-2Eβ u
H-2Eβ b
H-2Eβ f
H-2Eβ u,b,f
Drb1*0301
Drb1*1401
Drb1*1301
Drb1*1201
Drb1*11011
Drb1*0801
Drb4*0101
Drb7
Drb6*0101
Drb3*0101
Drb3*0201
Drb1*0101
Drb1*1001
Drb1*0401
Drb1*09012
Drb5*0101
Drb1*0701
Drb9
HLADRB1-9

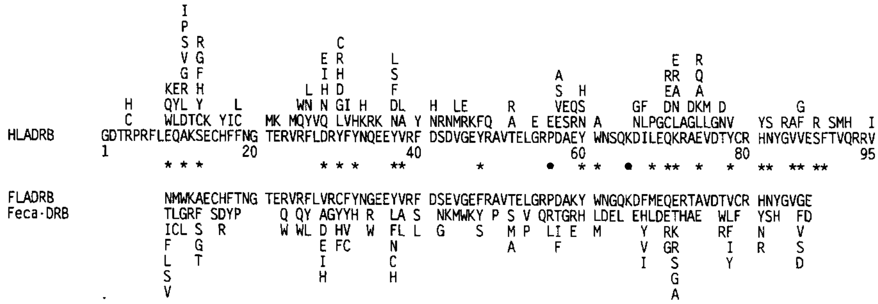


FIGURE 7. Distributions of highly polymorphic and conserved amino acid residues in β1 domain of feline and human *DRB* class II molecules. Consensus amino acid sequences in first extracellular domain of *DRB* class II molecule for human (*HLADRB*) and domestic cat (*FLADRB*) were shown. Each consensus amino acid sequence for *HLADRB* and *FLADRB* does not represent an individual molecule. ARS based on x-ray crystallographic analysis of human class II *HLA-DR1* molecule (24) are indicated by asterisks. Single letters above human and below cat *DRB* sequences represent polymorphic amino acid residues.

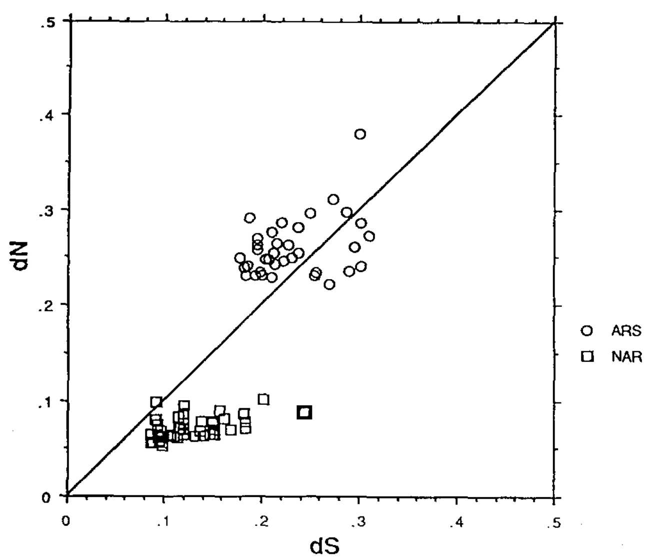


FIGURE 8. Synonymous (noncodon altering) and nonsynonymous (codon altering) nucleotide substitution rates in the ARS, NAR defined by an x-ray crystallographic structure of *HLA-DR1* class II Ags. Forty-nine *DRB* sequences were analyzed by pairwise comparisons using the method of Nei and Gojobori (23) to calculate dS and dN for their ARS (circles) and NAR (rectangles). Each symbol represents the average of all pairwise comparisons that measure the number of nonsynonymous or synonymous substitution/total number of sites for potential nonsynonymous or synonymous substitution for each sequence.

Table IV. *Number of DRB allelic sequences in mammals*

Species		No. of Individuals Studied	No. of *DRB* Alleles	Reference No.
Common name	Species name			
Human	*Homo sapiens*	251	126	33
Common chimpanzee	*Pan troglodytes*	64	85	35
Gorilla	*Gorilla gorilla*	19	40	35
Domestic cat	*Felis catus*	37	61	This study
Cattle	*Bos primigenius taurus, Bos primigenius indicus*	Not known	64	36–43
Red deer	*Cervus elaphus*	50	34	44
Pig	*Sus scrofa*	12	9	45, 46
Sheep	*Ovis aries*	15	13	47
Goat	*Capra hircus*	25	21	47
European moose	*Alces alces*	30	10	48
North American moose	*Alces alces*	19	10	48

Acknowledgments

The authors thank Dr. Fumiharu Shinyashiki, Okinawa International University, Ginowan, Okinawa, for his kind gift of *DRB* class II DNA fragments amplified by PCR from DNAs in Iriomote cat (*Mayailurus iriomotensis*); Dr. Colm O'hUgin, Max Planck Institute, for providing compiled data of primate *DRB* sequences; and Drs. Jill Slattery and J. Clay Stephens for useful suggestions for phylogenetic analysis. The authors also thank Leslie Wachter, Stanley Cevario, Mary Thompson, and Janice Martenson for their excellent technical assistance.

References

1. Klein, J. 1986. *Natural History of the Major Histocompatibility Complex.* John Wiley & Sons, New York.
2. Migaki, G. 1982. Section VIII, compendium of inherited metabolic diseases in animals. In *Animal Models of Inherited Metabolic Disease.* R. Desnick, D. Patterson, and D. G. Scarpelli, eds. Alan R. Liss, New York, p. 473.
3. Cotter, S. M., W. D. Hardy, Jr., and M. Essex. 1975. Association of feline leukemia virus with lymphosarcoma and other disorders in the cat. *J. Am. Vet. Med. Assoc. 166:449.*
4. Pedersen, N. C., E. W. Ho, M. L. Brown, and J. K. Yamamoto. 1987. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science 235:790.*
5. Pedersen, N. C. 1987. Feline infectious peritonitis virus. In *Virus Infections of Carnivores.* Vol I. M. J. Appel, ed. Elsevier, New York, pp. 267–286.
6. Janczewski, D. N., W. S. Modi, J. C. Stephens, and S. J. O'Brien. 1995. Molecular evolution of mitochondrial 12S RNA and cytochrome *b* sequences in the pantherine lineage of Felidae. *Mol. Biol. Evol. 12:690.*
7. O'Brien, S. J., J. S. Martenson, W. E. Johnson, S. Miththapala, D. N. Janczewski, J. P. Slattery, D. A. Gilbert, C. Packer, M. E. Roelke, M. Bush, and D. E. Wildt. 1996. Conservation genetics of the Felidae. In *Conservation Genetics of Rare and Endangered Species.* J. C. Avise and J. Harmick, eds. Chapman and Hall, New York, pp. 50–74.
8. Yuhki, N., and S. J. O'Brien. 1988. Molecular characterization and genetic mapping of class I and class II genes of the domestic cat. *Immunogenetics 27:414.*
9. Yuhki, N. 1995. The feline major histocompatibility complex. In *Feline Immunology and Immunodeficiency.* B. J. Willett and O. Jarrett, eds. Oxford University Press, Oxford, U.K., p. 45.
10. Winkler, C., A. Shultz, S. Cevario, and S. J. O'Brien. 1989. Genetic characterization of FLA, the cat major histocompatibility complex. *Proc. Natl. Acad. Sci. USA 86:943.*
11. Yuhki, N., and S. J. O'Brien. 1990. DNA variation of the mammalian major histocompatibility complex reflects genomic diversity and population history. *Proc. Natl. Acad. Sci. USA 87:836.*
12. Yuhki, N., G. F. Heidecker, and S. J. O'Brien. 1989. Characterization of MHC cDNA clones in the domestic cat: diversity and evolution of class I genes. *J. Immunol. 142:3676.*
13. Yuhki, N., and S. J. O'Brien. 1990. DNA recombination and natural selection pressure sustain genetic sequence diversity of the feline MHC class I genes. *J. Exp. Med. 172:621.*

14. Yuhki, N., and S. J. O'Brien. 1994. Exchanges of short polymorphic DNA segments predating speciation in feline major histocompatibility complex class I genes. *J. Mol. Evol. 39:22.*
15. Gubler, U., and B. J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. *Gene 25:263.*
16. Sekaly, R. P., C. Tonnelle, M. Strubin, B. Mach, and E. O. Long. 1986. Cell surface expression of class II histocompatibility antigens occurs in the absence of the invariant chain. *J. Exp. Med. 164:1490.*
17. Tonnelle, C., R. DeMars, and E. O. Long. 1985. DOB: a new B chain gene in HLA-D with a distinct regulation of expression. *EMBO J. 4:2839.*
18. Carrington, M., M. B. White, M. Dean, D. Mann, and F. E. Ward. 1992. The use of DNA heteroduplex patterns to map recombination within the HLA class II region. *Hum. Immunol. 33:114.*
19. Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the Vax. *Nucleic Acids Res. 12:387.*
20. Stephen, A. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol. 215:403.*
21. Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing evolutionary trees. *Mol. Biol. Evol. 4:406.*
22. Felsenstein, J. 1993. PHYLIP: phylogenetic inference package. Version 3.5c. University of Washington, Seattle, WA.
23. Nei, M., and T. Gojobori. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol. Biol. Evol. 3:418.*
24. Brown, J. H., T. S. Jardetzky, J. C. Gorga, L. J. Stern, R. G. Urban, J. L. Strominger, and D. C. Wiley. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature 364:33.*
25. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science 239:487.*
26. Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*
27. Klein, J., R. E. Bontrop, R. L. Dawkins, H. A. Erlich, U. B. Gyllensten, E. R. Heise, P. P. Jones, P. Parham, E. K. Wakeland, and D. I. Watkins. 1990. Nomenclature for the major histocompatibility complexes of different species: a proposal. *Immunogenetics 31:217.*
28. Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger, and D. C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature 329:506.*
29. Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger, and D. C. Wiley. 1987. The foreign antigen binding site and T-cell recognition regions of class I histocompatibility antigens. *Nature 329:512.*
30. Hughes, A. L., and M. Nei. 1988. Pattern of nucleotide substitution rate at major histocompatibility complex class I loci reveals overdominant selection. *Nature 335:167.*
31. Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. In *Mammalian Protein Metabolism.* H. N. Munro, ed. Academic Press, New York, p. 21.
32. Hughes, A. L., and M. Nei. 1989. Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. *Proc. Acad. Natl. Sci. USA 86:958.*
33. Bodmer, J. G., S. G. E. Marsh, E. D. Albert, W. F. Bodmer, B. Dupont, H. A. Erlich, B. Mach, W. R. Mayr, P. Parham, T. Sasazuki, G. M. T. Schreuder, J. L. Strominger, A. Svejgaard, P. I. Terasaki. 1994. Nomenclature for factors of the HLA system. *Tissue Antigens 44:1.*
34. Albright-Fraser, D. G., R. Reid, V. Gerber, and E. Bailey. 1996. Polymorphism of DRA among equids. *Immunogenetics 43:315.*
35. O'hUigin, C., R. Bontrop, and J. Klein. 1993. Nonhuman primate *Mhc-DRB* sequences a compilation. *Immunogenetics 38:165.*
36. Xu, A., M. J. T. van Eijk, C. Park, and H. A. Lewin. 1993. Polymorphism in *Bola-DRB* exon 2 correlates with resistance to persistent lymphocytosis caused by bovine leukemia virus. *J. Immunol. 151:6977.*
37. Groenen, M. A. M., J. J. van der Poel, R. J. M. Dijkhof, and M. J. Giphart. 1990. The nucleotide sequence of bovine MHC class II DQB and DRB genes. *Immunogenetics 31:37.*
38. Sigurdardottir, S., C. Borsch, K. Gustafsson, and L. Andersson. 1991. Cloning and sequence analysis of 14 DRB alleles of the bovine major histocompatibility complex by using the polymerase chain reaction. *Anim. Genet. 22:199.*
39. Burke, M. G., R. T. Stone, and N. E. Muggli-Cockett. 1991. Nucleotide sequence and Northern analysis of a bovine major histocompatibility class II DRB-like cDNA. *Anim. Genet. 22:343.*
40. Ammer, H., F-W. Schwaiger, C. Kammerbauer, M. Gomolka, A. Arriens, D. Lazary, and J. T. Epplen. 1992. Exonic polymorphism vs intronic simple repeat hypervariability in *MHC-DRB* genes. *Immunogenetics 35:332.*
41. Gelhaus A., L. Schnittger, D. Mehlitz, R. D. Horstmann, and C. G. Meyer. 1995. Sequence and PCR-RFLP analysis of 14 novel *Bola-DRB3* alleles. *Anim. Genet. 26:147.*
42. Russell, G. C., K. L. Marello, A. Gallagher, D. J. McKeever, and R. L. Spooner. 1994. Amplification and sequencing of expressed DRB second exons from *Bos indicus. Immunogenetics 39:432.*
43. Mikko, S., and L. Andersson. 1995. Extensive MHC class II DRB3 diversity in African and European cattle. *Immunogenetics 42:408.*
44. Swarbrick, P. A., F. W. Schwaiger, J. T. Epplen, G. S. Buchan, J. F. T. Griffin, and A. M. Crawford. 1995. Cloning and sequencing of expressed *DRB* genes of the red deer *(Cervus elaphus) Mhc. Immunogenetics 42:1.*
45. Gustafsson, K., S. Germana, F. Hirsh, K. Pratt, C. LeGuern, and D. H. Sachs. 1990. Structure of miniature swine class II *DRB* genes: conservation of hypervariable amino acid residues between distantly related mammalian species. *Proc. Natl. Acad. Sci. USA 87:9798.*
46. Vage, D. I., I. Olsaker, F. Lingaas, and O. Lie. 1994. Isolation and sequence determination of porcine class II DRB alleles amplified by PCR. *Anim. Genetics 25:73.*
47. Shwaiger, F-W., E. Weyers, C. Epplen, J. Brun, G. Ruff, A. Crawford, and J. T. Epplen. 1993. The paradox of *MHC-DRB* exon/intron evolution: α-helix and β-sheet encoding regions diverge while hypervariable intronic simple repeats coevolve with β-sheet codons. *J. Mol. Evol. 37:260.*
48. Mikko, S., and L. Andersson. 1995. Low major histocompatibility complex class II diversity in European and North American moose. *Proc. Natl. Acad. Sci. USA 92:4259.*